USDA ARS National Animal Germplasm Program

Swine Oocyte Collection, Transportation, Maturation and Vitrification Protocol

Collection and maturation:

Aspirate cumulus oocyte complexes (COC) from 2 to 6 mm follicles and placed in groups of 50 in 400 µL in In Vitro Maturation Medium.

Incubate COCs at 38.5 °C in 5% CO_2 for 44-46 hr and then graded. Only retain those oocytes classified as grade I (homogeneous cytoplasm and at least 3 layers of cumulus cells) or grade II (homogeneous cytoplasm and 1 or 2 layers of cumulus cells) for vitrification.

Gently pipette the acceptable oocytes to remove cumulus cells.

Transfer the acceptable oocytes to 39 °C Equilibration Solution. Oocytes can be held in this solution from 5 to 15 min.

Transfer oocytes to the Vitrification Solution and incubate for 20 to 30 s.

Place the ooctyes on a vitrification device (e.g. Cryotop from Kitazato Corp, Tokyo, Japan or Vitringa from INGÁMED, Maringá, PR, Brazil) and plunge into liquid nitrogen. The time from exposure to vitrification solution to plunging in liquid nitrogen is 45 to 60 s.

Thawing:

Thaw oocytes by plunging the devices containing oocytes into 39 °C Thawing Solution for 1 min.

Transfer the device/oocytes to Dilution Solution for 3 min.

Wash the oocytes through 2 separate rinses with Washing Solution.

Culture for IVF.

Recipes:

In Vitro Maturation Medium
80% TCM 199, 10% FBS, 10% Pig follicular fluid, 10 IU/mL eCG, 5 IU/mL hCG. *Note: the in vitro maturation medium is prepared without 10% pig follicular fluid which is added, after filtration, at the time of aspiration of the oocytes.*

Equilibration Solution
65% TCM 199, 7.5% ethylene glycol, 7.5% DMSO, 20% FBS (the manuscript uses synthetic serum substitute)

Vitrification Solution
15% ethylene glycol, 15% DMSO, 20% FBS, 0.5 M sucrose, TCM 199 to volume

Thawing Solution
1.0 M sucrose in TCM 199 with 20% FBS

Dilution solution
0.5 M sucrose in TCM 199 with 20% FBS

Washing Solution
TCM 199 with 20% FBS

Reference:

Liu, Y., Du, Y., Lin, L., Li, J., Kragh, P.M., Kuwayama, M., Bolund, L., Yang, H., Vajta, G. 2008. Comparison of efficiency of open pulled straw (OPS) and cryotop vitrification for cryopreservation of in vitro matured pig oocytes. Cryo-Letters 29: 315-320.

Versions: April 2017, April 2020.